The Effect of Low-Level Laser Radiation on Some Traits of Wheat (Triticum aestivum L.) Under Drought Stress

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Research Article

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Abstract

Wheat (*Triticum aestivum* L.), a major global staple crop, is highly vulnerable to drought stress, which substantially constrains growth and yield. Emerging evidence suggests that low-level laser irradiation may serve as an effective priming strategy to enhance plant tolerance to environmental stresses. In the present study, wheat seeds were pre-treated with low-level laser radiation (740 nm) for different exposure durations (0, 45, 65, and 85 s) and subsequently grown under controlled pot conditions subjected to two irrigation regimes (80% and 40% field capacity). Laser pre-treatment for 45 and 65 s significantly improved morphological and physiological traits, including plant height, tiller number, node number, carotenoid content, and proline accumulation. Notably, laser-treated plants exhibited enhanced tolerance to drought stress, with pronounced improvements in both growth-related and biochemical parameters compared with untreated controls. Optimal laser exposure (~ 60 s) on wheat dry seeds appeared to promote dry weight of arial part of plant in no-stress condition and alleviate detrimental drought stress effect, sink-related traits, such as tiller and node formation, potentially through modulation of proline metabolism biosynthesis, which function as key osmoprotective and antioxidant mechanisms. This priming-like phenomenon is probable attributed to epigenetic changes in expression of mechanisms involved in free radicals scavenging, specially under drought stress, to alleviate the stress effects as a results of laser pre-treatment.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, serving as a vital food source for nearly one-third of the global population. Wheat's widespread cultivation is supported by favorable growing conditions and its rich nutritional content, including carbohydrates, proteins, fibers, vitamins, and minerals. However, wheat production is increasingly threatened by environmental stresses, such as water shortage, heat and salinity with drought being the most significant factor causing yield reductions (Rasheed et al., 2023; Shewry, 2009).

Energy require for plant growth and development is provided through cellular respiration, which reactive oxygen species (ROS) are usually produced as by-products (Palma et al., 2024). Drought stress induces oxidative stress in plants due to the overaccumulation of ROS, which, although serve as signaling molecules in low concentration (Schieber & Chandel, 2014), causes damage to cellular structures such as proteins, lipids, and membranes (Asada et al., 1999). To mitigate oxidative damage, plants rely on antioxidant defense systems, which include enzymatic ROS scavenging through enzymes like catalase and ascorbate peroxidase, as well as non-enzymatic mechanisms involving external protective agents (Asada et al., 1999).

Several approaches have been employed to improve plant resilience to environmental stresses, such as conventional breeding, genetic engineering, and chemical treatments (Dixit et al., 2014; Paul et al., 2023). In recent years, low-level laser treatment has garnered attention as a potential tool for enhancing plant stress tolerance. Laser treatments have been shown to activate stress resistance mechanisms, enhance

photosynthesis, and boost ATP production in plants, ultimately leading to improved stress tolerance (Aslam et al., 2022; Qiu et al., 2013).

Laser technology, first introduced by Einstein in 1917, operates through coherent, monochromatic light, distinguishing it from other light sources (Hecht, 2018)). Low-power lasers (characterized by low input power and specific frequency ranges) are increasingly being explored for agricultural applications, including stress alleviation and crop performance enhancement (Vuylsteke & Mordon, 2012). Despite promising results, most laser treatments have focused on soaked or moist seeds, which poses challenges for large-scale adoption in automated planting systems.

The objective of this study, is to explores the effect of low-power laser pretreatment on dry wheat seeds and examines its potential to enhance drought tolerance under controlled conditions. To this end we evaluated effects of dry seed low-level laser irradiation on some morphological, physiological, and biochemical attributes of adult wheat plant and tried to find the possible underlying mechanisms.

Materials and Methods

Seed Material and Cultivation

Seeds of *Triticum aestivum* L. (Baharan cultivar) were obtained from the Seed and Plant Improvement Institute, Karaj, Iran. These seeds were planted in pots containing a mixture of 2.5 kg sandy clay loam and decomposed manure (2:1 ratio, w/w). Three seeds were planted per pot, later thinned to one plant per pot. The pots were placed in a greenhouse with controlled temperature (25°C day/20°C night) and light intensity (4151–5500 lux) under a 16-hour light/8-hour dark photoperiod. Plants were fertilized twice with an NPK solution (2 g per liter of water) during the vegetative stage.

Experimental Treatments

The experiment followed a completely randomized design (CRD) with a factorial arrangement and three replications. The factors included laser radiation duration (4 levels: 0, 45, 65 and 85 sec) and irrigation regimes (two levels: 80% field capacity and 40% field capacity). The laser treatment was administered using a LAZAR-F laser device (Plant Mode laser, LAZAR-Co, Karaj, Iran), emitting 740 nm laser light at 500 mW power and 80 Hz frequency. Dry seeds were pre-treated for different durations (0, 45, 65, and 85 seconds) from a distance of 1 cm, while control seeds were not irradiated.

Irrigation Stress

Irrigation treatments were applied beginning at the five-leaf stage, with two water regimes: 80% field capacity (non-stress) and 40% field capacity (drought stress). Drought stress was applied until the 8-leaf stage and the plants were normally irrigated, thereafter.

Sampling and Growth Parameter Measurements

At the 8-leaf stage, the above-ground parts of the plants were harvested. Plant height was measured from the soil surface to the top of the stem, and leaf area per plant was assessed using a digital leaf area meter (Licor 3100, USA). Additional growth parameters, including tiller number, node count, and leaf dimensions (length and width), were also measured. The plants were oven-dried at 70°C for dry weight determination.

Biochemical Analysis

Frozen leaf tissue was homogenized in liquid nitrogen and stored at -80°C for later biochemical analyses. The concentrations of chlorophylls a, b and carotenoids were determined spectrophotometrically following Zhou et al (2019). Proline content was measured using the method of Bates et al. (1373), and ascorbate peroxidase activity was assessed according to Nakano & Asada (1981).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in RStudio (Version 2021). Mean comparisons were made using Duncan's multiple range test at a significance level of $\alpha \leq 0.05$ Graphs were generated using Excel 2021.

Results

Analysis of variance was performed on according to the CRD arrangement (Table 1).

Table 1
ANOVA results on the data obtained in the experiment in the frame od CRD arrangement

Source of Variation	DF	Mean Squares					
		Plant Height	No.of tillers	No.of nodes	Leaf length	Leaf width	Dry weight of shoot
Laser radiation(L)	3	140.71**	1.59**	2.69**	985.59**	19.98**	0.09**
Drought stress (S)	1	10482.4**	3.33**	15.55**	101588.9**	198.20**	0.58**
L×S	3	26.73*	0.26**	0.47**	251.61*	3.34*	0.009**
CV (%)		6.30	9.36	8.49	6.68	6.76	4.53
* and ** stand for significant effect at $\alpha \leq 0.05$ and $\alpha \leq 0.01$, respectively.							

Table 1
continued

Source of Variation	DF	Mean Squares				
		Chlorophyll a	Chlorophyll b	Carotenoids	APX	Proline
Laser Radiation(L)	3	0.18**	0.03**	0.07**	9.69**	7.16**
Drought Stress (S)	1	7.55**	0.98	0.09**	153.43**	45.63**
L×S	3	0.31**	0.03*	0.01**	4.90**	4.74**
CV (%)	-	5.13	7.80	6.85	7.35	8.02
* and ** stand for significant effect at $\alpha \leq 0.05$ and $\alpha \leq 0.01$, respectively.						

The coefficient of variation (CV) for all evaluated traits was less than 10%, ensuring the reliability of the data collected. As shown in Table 1, the interaction between laser radiation and drought stress was significant for all studied traits at either $\alpha \leq 0.05$ or $\alpha \leq 0.01$. Therefore, mean comparisons were conducted only for the interaction effects.

Growth Parameters

Pre-treating seeds with laser irradiation for 45 sec significantly increased plant height under non-stress conditions. Laser exposure for 45 and 65 sec had similar effects on plant height. However, increasing the exposure time to 85 sec resulted in a significant decrease in plant height (Fig. 1-a).

Drought stress significantly reduced plant height. Under drought condition, laser irradiation for 45 and 65 sec similarly and significantly increased plant height. However, extending the exposure time to 85 sec reduced plant height to levels comparable to those observed in the non-irradiated control (Fig. 1-a).

Leaf dimensions (length and width) were differentially influenced by laser exposure time.Under non-stress condition, exposure times of 45 and 65 sec significantly increased both leaf length and width. However, extending the exposure to 85 sec returned leaf length to values comparable to the non-irradiated control, while leaf width was reduced to significantly lower levels than in un-treated plants (Fig. 1-b and c).

Drought stress significantly reduced both leaf length and width. Seed pre-treatment with lasers at various exposure times, except for 65 sec, did not affect leaf length under drought conditions. However, leaf width was significantly increased with laser exposure times of 45 and 65 sec. An exposure time of 85 sec caused a significant decrease in leaf width under both non-stress and drought conditions (Fig. 1-b and c).

Under non-stress conditions, seed pre-treatment with laser irradiation significantly increased the number of nodes per plant, with 45 and 65 sec of exposure producing similar results. Although exposure times

longer than 65 sec led to a significant reduction in node number, the values remained higher than those observed in plants that did not receive laser treatment (Fig. 1-d).

Drought stress had a significant negative effect on the number of nodes per plant. Laser radiation significantly increased the number of nodes, with the highest count observed at 65 sec of exposure. Laser treatments of 65 and 85 sec effectively mitigated the negative impact of drought stress on node number. However, exposure times longer than 65 sec resulted in a significant reduction in node number, although the values remained higher than those observed in plants that did not receive laser treatment (Fig. 1-d).

Under non-stress conditions, laser pre-treatment of seeds for 45 and 65 sec significantly increased the number of tillers per plant. However, laser exposure for 85 sec resulted in a tiller number comparable to that of the non-irradiated control (Fig. 1-e).

Drought stress significantly reduced the number of tillers per plant. However, laser radiation for 65 sec completely mitigated the negative effects of stress on tiller number. A biphasic trend was observed under stress conditions: increasing the exposure time to 85 sec resulted in a significant reduction in the number of tillers per plant compared to the 65-sec treatment, although the tiller number remained significantly higher than in plants that did not receive laser treatment (Fig. 1-e).

Under non-stress conditions, pre-treating seeds with laser irradiation for 45 or 65 sec equally and significantly increased above-ground biomass. However, extending the irradiation time beyond this point resulted in a significant reduction in above-ground biomass (Fig. 1-f).

Drought stress significantly reduced the dry mass. Nevertheless, this reduction was partially mitigated when seeds were irradiated with laser for 45 or 65 sec (Fig. 1-f).

Photosynthesis apparatus

No significant changes were observed in chlorophyll a and b contents in non-stressed plants when seeds were pre-treated with lasers at different exposure times. In contrast, drought stress had a significant impact on the levels of both chlorophyll a and b (Fig. 2).

Under drought stress, pre-treating seeds with laser exposure for 45 and 65 sec resulted in significant increases in both chlorophyll a and b concentrations. However, extending the laser exposure by an additional 20 sec caused a significant decrease in the levels of both chlorophyll types.

Under non-stress conditions, laser radiation at all exposure times except for 85 sec did not significantly affect carotenoid content; however, irradiation for 85 seconds significantly reduced carotenoid content.

Under non-stress conditions, laser radiation at all exposure times except for 85 sec did not significantly affect carotenoid content; however, irradiation for 85 seconds significantly reduced carotenoid content.

Drought stress led to a significant reduction in carotenoid content. Under drought conditions, laser exposure for 45 and 65 sec significantly increased carotenoid levels, but extending the exposure by an additional 20 sec resulted in a sharp decline. Notably, a 65-sec laser treatment completely mitigated the effects of drought stress on carotenoid content, restoring it to levels comparable to those observed under non-stress conditions (Fig. 2).

Ascorbat peroxidase activity

Under non-stress conditions, the activity of ascorbate peroxidase (APX) in response to laser radiation exhibited a time-dependent pattern. Laser exposure for 45 sec did not affect enzyme activity compared to the untreated control. Nevertheless, APX activity significantly increased after 65 sec of irradiation. When the exposure was extended by an additional 20 sec beyond 65 sec, the enzyme activity significantly decreased to levels below those observed in the non-irradiated condition (Fig. 3).

Drought stress significantly increased APX activity. Pre-treating seeds with laser irradiation for 45 and 65 seconds progressively enhanced the enzyme activity; however, extending the exposure time to 85 sec reduced the activity back to the level observed after 45 sec of treatment (Fig. 3).

Proline Content

Seed pre-treatment with laser irradiation for 65 and 85 sec significantly increased proline content under non-stress conditions, reaching levels comparable to those observed under drought stress. Drought stress alone also significantly elevated proline content. Laser pre-treatment further enhanced proline accumulation, with the highest proline levels recorded in seeds exposed to both drought stress and 65 sec of laser radiation, followed by those treated for 45 sec under the same conditions. Additionally, seeds pre-treated with laser for 85 sec and subjected to drought stress showed a significant increase in proline content compared to the non-irradiated control (Fig. 4).

Discussion

The signaling effects of low-level lasers in animal science have been reported by several researchers (Gao & Xing, 2009; Tamimi et al., 2025; Yin et al., 2017). Nevertheless, due to the absence of a circulatory system and nervous network in plants, the systemic mechanism of laser radiation effect at the whole-plant level remains unclear.

In this investigation, we examined the effects of pre-treating wheat seeds with low-level laser irradiation on various attributes of the wheat plant. While many studies have focused on the impact of different types of laser pre-treatments on the biophysical properties of seeds and their germination (Aftab & Younus, 2020; AlSalhi et al., 2018; Chervinsky et al., 2022), there is growing evidence that laser-induced effects on seeds can be transmitted through to the seedling and mature plant stages (Chen et al., 2010; Qiu et al., 2008; Qiu et al., 2013).

This experiment was conducted under two irrigation regimes: well-watered (non-stress) and drought stress. In both regimes, dry wheat seeds were exposed to low level laser irradiation for 45, 65, and 85 sec, and subsequently cultivated in pots until physiological maturity. In the non-stress condition, irrigation was applied regularly throughout the growth period until maturity to provide 80% of field capacity. In the drought stress condition, irrigation was restricted to 45% of field capacity from the 5-leaf to the 8-leaf stage and retuned back to the normal condition, thereafter.

Under the both conditions, the responses of selected morphological, physiological, and biochemical traits with special focus on plant dry weigh to laser pre-treatment were assessed.

Normal condition

Two opposite routs, photosynthesis and respiration, are involved in formation of dry wight in plants. Their relevant rates and impacts over each other finally determine the plant dry weight

The rate of photosynthesis is constrained by two main categories of factors: stomatal and non-stomatal limitations. Stomatal limitation refers to a reduction in photosynthetic capacity resulting from decreased stomatal aperture, which restricts CO_2 influx and thereby suppresses plant growth and development (Assmann, 1988). In contrast, non-stomatal limitation arises from impairments in biochemical processes, such as reduced Rubisco activity or altered chlorophyll content (Perdomo et al., 2017). The absence of significant changes in chlorophyll concentration following laser pre-treatment suggests that the observed effects of laser irradiation on traits such as dry weight are likely to be driven in part by nonstomatal mechanisms.

Although laser pre-treatment did not increase photosynthetic biochemical factors such as chlorophyll a, chlorophyll b, or carotenoid content, the observed enhancements in biomass and leaf size may be caused either through improved photosynthetic efficiency(Kumar et al., 2010) or reduction of ROS production (Qi et al., 2025) or

Laser irradiation enhanced plant biomass, leaf dimensions, and the number of tillers and nodes. Similar effects of laser treatment on tiller induction have been reported by other researchers (Aslam et al., 2022; Drozd, 1994; Koper, 1994). Tillering patterns are also known to be regulated by a carotenoid-derived compound, strigolactone which modulates the auxin/cytokinin balance that controls tiller formation ((Assuero & Tognetti, 2010; Stirnberg et al., 2010). This relationship become particularly evident in the present study, as the reduction in tiller number observed under 85 s of laser exposure coincided with a decline in carotenoid content. Accordingly, we detected concomitant changes in carotenoid concentration and tiller number as a function of laser exposure time, consistent with the findings of (Hernandez Aguilar et al., 2008).

In wheat, tiller characteristics- including number and angle-are influenced by several factors, such as population density, nitrogen and assimilate availability, phytohormones (auxin, cytokinins, abscisic acid), temperature, and salinity (Shaheen et al., 2025). The changes in tiller number following laser irradiation

may therefore result from laser-induced modulation of auxin biosynthesis or signaling (Aslam et al., 2022).

Tillers originate from nodes (meristems) on the crown (Gao et al., 2019) thus, both stem node number and tiller production are likely governed by overlapping factors, including auxin levels (Aslam et al., 2022) and assimilate supply. Although laser pretreatment did not significantly affect plant height, it markedly increased node number, and thus reducing average internode length. Because internodes serve as sites for non-structural carbohydrate storage (Mostafaie et al., 2025), this anatomical shift likely contributes to a greater density of stored reserves.

While significant increases in plant height, leaf area and shoot dry weight was reported in seed wheat pre-treated by low level laser at 630nm for 1min, no change reported in tiller number and carotenoids content (Aslam et al., 2022). The effects were unpredictably increased/decreased up on prolonged radiation exposure time (Aslam et al., 2022).

Biological systems such as photosynthetic apparatus is continually exposed to reactive oxygen species (ROS), or free radicals, which are routinely generated during cellular respiration. Plants counteract this oxidative stress through an antioxidant system that scavenges ROS. In the present study, both ascorbate peroxidase (APX) activity and proline content increased when wheat seeds were pre-treated with laser irradiation for 65s. Numerous studies have demonstrated that proline protects cellular structures and stabilizes enzymes involved in antioxidant defense (Mohammadrezakhani et al., 2019; Naliwajski & Skłodowska, 2021; Singh et al., 2025). APX, a key enzyme in hydrogen peroxide (H_2O_2) metabolism, reduces H_2O_2 to water using ascorbate (ASC) as an electron donor in various subcellular compartments (Bunkelmann & Trelease, 1996; Yamaguchi et al., 1995; Yoshimura et al., 1999). Moreover, APX has been implicated in lignin biosynthesis in *Brachypodium distachyon* and Arabidopsis (Barros et al., 2019), which may explain the higher aerial biomass observed after 65s of laser treatment, consistent with the APX response.

The influence of exposure duration was evident: extending irradiation from 65s to 85s resulted in a significant decline in APX activity. Efficient antioxidant functioning minimizes ROS-induced damage and lowers maintenance respiration, thereby reducing the consumption of stored reserves. This conservation of resources, combined with increased tiller number and the accumulation of assimilates in internodes, likely contributed to the observed rise in dry weight (Fig. 1-f).

Previous research has similarly reported significant increases in plant height, leaf area, and shoot dry weight in wheat seeds pre-treated with low-level red laser light (630nm) for 1 min, without accompanying changes in tiller number or carotenoid content (Aslam et al., 2022). Prolonged irradiation, however, produced variable outcomes, with both increases and decreases depending on exposure time (Aslam et al., 2022).

While no significant effects of irradiation on the activity of photosynthesis-related biochemical components were observed, laser irradiation for 65 sec significantly increased the activity of ascorbate

peroxidase (APX). Scavenging free radicals reduces the risk of nucleophilic attack on biological macromolecules such as plasma membranes, photosynthesis apparatuses, and DNA. In contrast to our findings, Aslam et al. (2022) reported no significant changes in enzyme activity when wheat seeds were pre-treated with a low-level Ne-He laser at 630 nm for 1, 2, and 3 minutes. Moreover, Al-Quraan, et al (2022) reported that blue-wavelength laser irradiation of wheat and lentil leaves for 20, 40, or 60 minutes decreased chlorophyll a and b levels (Al-Quraan et al., 2022), These discrepancies may be due to two main factors: exposure time and laser type. Lasers typically exhibit three biological effects: the photophysical effect, which causes tissue heating; the photochemical effect, which interacts with electrons; and the photovoltaic effect. Depending on the laser type and exposure time, these effects can vary significantly.

The phenotypes observed in the adult plant up on seed pre-treating with laser radiation may be caused by either genetics or epigenetics effects. In this investigation, the lasers used were classified as low-level lasers based on their energy characteristics. Their low frequency and high wavelength categorize them as safe therapeutic tools for humans (Farivar et al., 2014), effectively nullifying any mutagenic effects. Furthermore, the lasers were applied to dried seeds, which exhibited minimal metabolic activity (El-Maarouf-Bouteau, 2022),

Providing the short half time of macromolecules such as proteins and hormones, it is almost unlike that the laser radiation at seed stage causes production of special proteins or hormones which transmit to the adult plant to cause the observed phenotypes. Thus for, we may hypothesize presenting of a temporary gene regulation story, *i e* epigenetic phenomenon. The number of nodes (node number per plant) in wheat is primarily controlled by genetic factors, although environmental conditions also play a role (Shang et al., 2021). Under normal field conditions with adequate water supply, wheat plants typically generate about 5 nodes per plant (Shang et al., 2021). Laser pre-treatment of wheat seeds for 45 and 65 sec significantly induced an increase in the number of nodes compared to the non-radiated stat,. On the other hand, the seeds, after laser pre-treatment, and the resulting seedlings were maintained under the same conditions, thereby ruling out any potential environmental effects on the number of nodes. Thus for, it may be concluded that the observed phenotypes in adult plant when the seeds pre-treated with lase radiation may have been governed by epigenetic mechanisms.

Drought stress condition

All the evaluated morphological traits were reduced under drought stress. It was in contrary to what was reported by Aslam et al. (2022). They reported no significant reduction for all the morphological traits. Aside from different genetic background, this discrepancy may be caused by stress severity which was 40% FC in the present investigation versus 50% FC in their experiment

In plants, growth is defined as a permanent increase in size and volume resulting from cell division and expansion (Hilty et al., 2021). Drought stress typically reduces leaf dimensions, which may be attributed to decreased cell division, reduced cell volume, or both (Skirycz et al., 2011). However, under drought conditions, laser pre-treatment was found to increase leaf dimensions -particularly leaf width- compared

to the control group. This enhancement is likely more attributable to increased cell division than to cell enlargement. Induction of cell division via laser radiation has been frequently reported in both animal (Ganjali et al., 2018) and plants (Metwally et al., 2013).

The effect of seed-pretreating with laser radiation on leaf dimensions exhibited a dose-dependent response. Previous studies in animals (Ganjali et al., 2018), bacteria (Chung et al., 2014), and plants (Metwally et al., 2020) have demonstrated that the cellular response to laser irradiation follows a biphasic dose-dependent pattern: low doses stimulate proliferation, while higher doses can inhibit cell proliferation or damages it. All the investigated traits showed similar response nder drought conditions.

Plant height is determined by the interaction between the number of nodes and internode length. Therefore, drought stress, by reducing internode length (a consequence of decreased turgor pressure and inhibited cell division) and decreasing the number of nodes, results in reduced plant height (Fig. 1-a). laser application for 65 sec increased plant height and the number of nodes by 41% and 67%, respectively, compared to the non-irradiated control group. The greater plant height observed in the 65-sec laser pre-treated plants under drought stress may be attributed to enhanced node formation or increased internode length through stimulated cell division. The effectiveness of low-level lasers in mitigating drought-induced reductions in plant height has also been reported in *Celosia argentea*, where a biphasic, dose-dependent response of plant height to laser treatment was observed, as well (Ali et al., 2020).

Drought stress caused a significant reduction in tiller number. Yet, as under non-stress conditions, laser pre-treatment of seeds increased tiller number in a biphasic dose-dependent manner. Notably, a 65 sec laser exposure fully counteracted the drought-induced decline in tiller number. In contrast, the same treatment did not offset the drought effect on node number, suggesting that node formation is regulated by more complex mechanisms than tiller initiation. This difference may partly reflect the stimulatory influence of laser irradiation on the biosynthesis of carotenoids and phytohormones such as auxins and cytokinins (Aslam et al., 2022).

Tiller production represents a key compensatory response to adverse conditions (Mmbando, 2025), helping to maintain yield. Laser irradiation for 65s effectively alleviated the drought-related reduction in tiller number and likewise mitigated the associated decrease in dry weight.

Carotenoids are antioxidants that are able to detoxify various forms of ROS and also directly quench triplet chlorophylls that are sources of $1O_2$ in leaves (Ramel et al., 2012). Decreases in carotenoid content under drought stress conditions has been reported in wheat (Hammad & Ali, 2014) and sorghum (Arivalagan & Somasundaram, 2015) which was in align with the present investigation.

An increase in carotenoid content in plants derived from laser pre-treated seeds may reflect an enhanced reactive oxygen species (ROS) scavenging system. The higher activity of protective and antioxidant mechanisms in plants subjected to drought stress up on laser pre-treatment, combined with the absence of adverse effects of the laser irradiation on photosynthetic biochemistry (specifically chlorophyll

content) under drought conditions, likely contributes to improved biomass accumulation by regulating maintenance respiration.

Reduced water availability is detrimental to photosynthetic capacity, primarily by inducing stomatal closure and limiting carbon fixation, but it also generates oxidative stress through the overproduction of ROS. Although ROS are natural by-products of normal processes such as respiration and photosynthesis, their accumulation under abiotic or biotic stress conditions causes cellular oxidative damage (Kotchoni et al., 2006). APX, along with other key antioxidant enzymes, plays a pivotal role in detoxifying ROS and safeguarding cellular function.

Drought stress markedly increased proline accumulation and ascorbate peroxidase (APX) activity. Similarly, 65 sec laser irradiation significantly enhanced these traits, suggesting that drought stress and laser treatment act synergistically to promote proline accumulation and APX activity. APX is recognized as a central component of the antioxidant system with a critical protective role under various abiotic stresses (Caverzan et al., 2012).

An increase in laser dose under drought conditions, achieved by prolonging irradiation time, further elevated proline accumulation and improved stress tolerance, as evidenced by an increase in dry weight. However, beyond a certain threshold, prolonged laser exposure reduced proline content, resembling the inhibitory effects on the dry weight observed with excessive proline supplementation (Satheeshkumar, 2024). This reduction may be mediated by methylation of proline biosynthesis genes. While proline-induced epigenetic reprogramming has been documented in plants (Satheeshkumar, 2024), such mechanisms have not yet been identified in response to laser irradiation. The differential effects of varying laser doses on proline accumulation may therefore be linked to changes in the methylation status of the promoter regions of genes controlling proline production.

Proline accumulation under drought stress is a general and widely reported adaptive response in plants exposed to different abiotic stresses(Szabados & Savouré, 2010), including drought conditions(Choudhary et al., 2005; Kamran et al., 2009). Our findings indicate that laser pretreatment of seeds can mimic drought-associated signaling through enhancing proline accumulation, thereby improving plant tolerance to water deficit. In this respect, laser pre-treatment may function analogously to the exogenous application of proline, which has been reported to alleviate salt stress in rice (Demiral & Türkan, 2005; Roy et al., 1993). Beyond its osmotic adjustment function, proline provides additional protection under stress by stabilizing proteins, maintaining redox balance, and scavenging free radicals (Slabbert & Krüger, 2014). Thus, the combined induction of APX activity and proline accumulation by drought and laser treatment represents a coordinated defense mechanism that enhances plant resilience to abiotic stresses.

Exposure of dry seeds to laser radiation prior to water imbibition may elicit a cascade of physiological and molecular responses that persist into subsequent developmental stages, a phenomenon collectively referred to as seed priming (Banisharif & Amooaghaie, 2025). Priming effects may arise from alterations in developmental timing (Hassan et al., 2024) and/or stable epigenetic modifications (Turgut-Kara et al.,

2020). Environmental stresses are well known to induce changes in DNA methylation patterns in plants (Qiao et al., 2024), and alterations in DNA methylation following low-dose laser irradiation have also been reported (Wang et al., 2010).

Although previous studies have reported laser-induced changes in gene expression and associated molecular pathways in wheat (Qiu et al., 2008), comprehensive transcriptomic analyses combined with detailed epigenetic profiling are still required to robustly validate this proposed mechanism.

Tthe synergistic effects observed between laser pre-treatment of wheat seeds and drought stress, particularly with respect to enhanced proline accumulation, are likely mediated by epigenetic regulatory mechanisms. These findings support the designation of this priming phenomenon as electromagnetic-mediated priming, herein referred to as electro-priming. Due to ease in application for large scale of treating dry seeds with the laser radiation, this technology may be used in large scale wheat production, worldwide/

Conclusion

Seed pre-treatment with low level laser of 80 HZ for 65 sec did not impose significant effect on photosynthetic apparatus. Nevertheless, the laser probably caused elevation in dry weight and its components such as tiller per plant and node per stem through epigenetic changes on the of antioxidant expression systems. This, in turn, may change temporal expression of genes involved in ROS scavenging pathways, which prevent or attenuate damages to biological systems. This protecting effect is more prominent under drought stress condition.

Declarations

Author Contribution

Y.M performed the experiment, analyzed the data, and prepared the first manuscript body and figuresM.S.N supervised the experiment and final checked the manuscript textA.R.A and A.H.H prepared the infrastructural need for laboratory testsA.L managed the radiation treatmentAll authors reviewed the manuscript

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Data Availability

We performed the experiment and generated data. The Dara would be available upon request.

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Figures

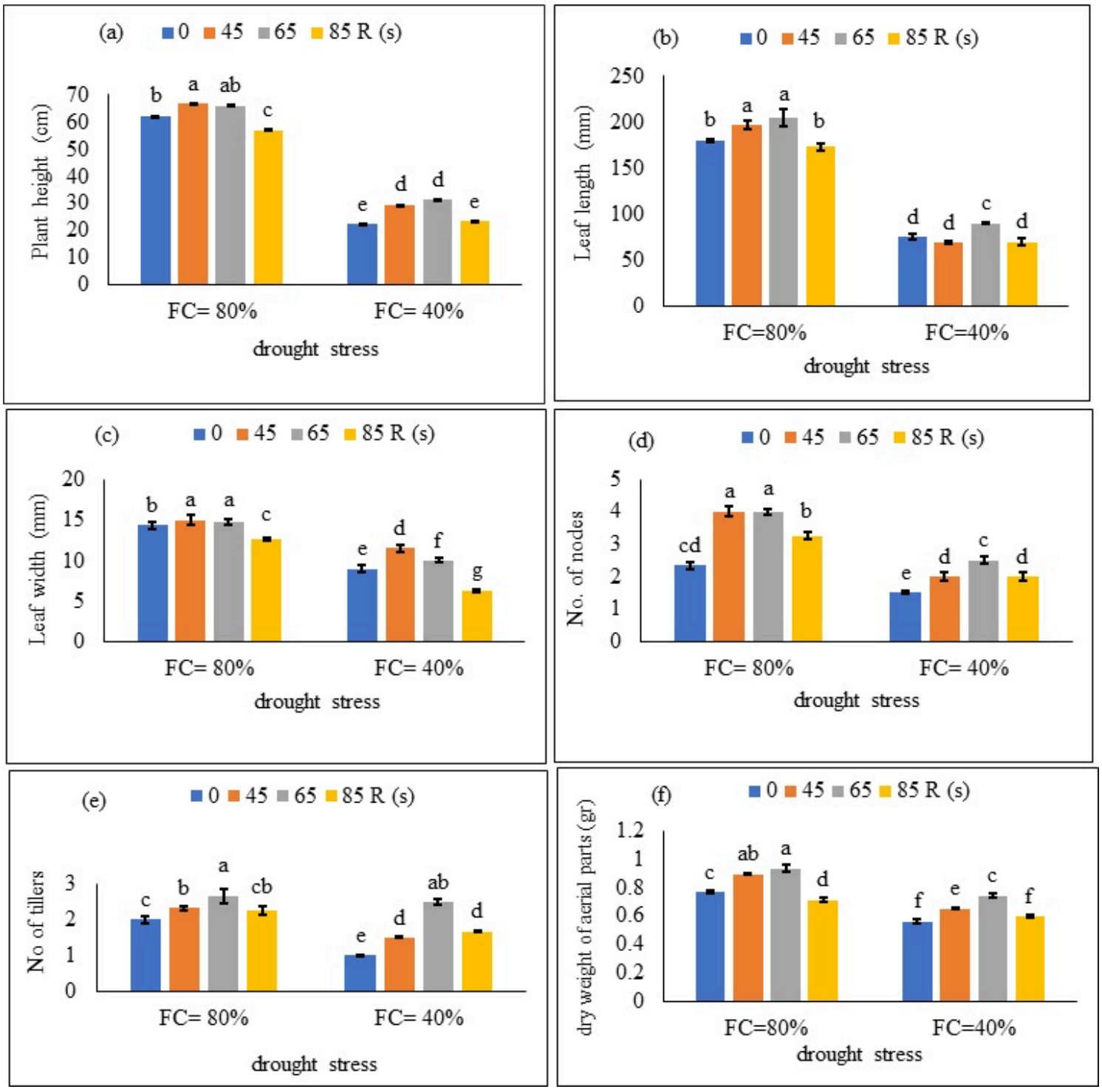

Figure 1

Comparison of the average interaction effect of laser radiation and drought stress in wheat on (a) plant height, (b) leaf length, (c) leaf width, (d) number of nodes, (e) number of tillers, (f) dry weight of aerial parts. Legend: R= laser radiation per sec, FC=field capacity. Bars with at least one letter in common do not have a statistically significant difference based on Duncan's test at the $p \leq 0.05\%$ or $p \leq 0.01\%$ probability level.

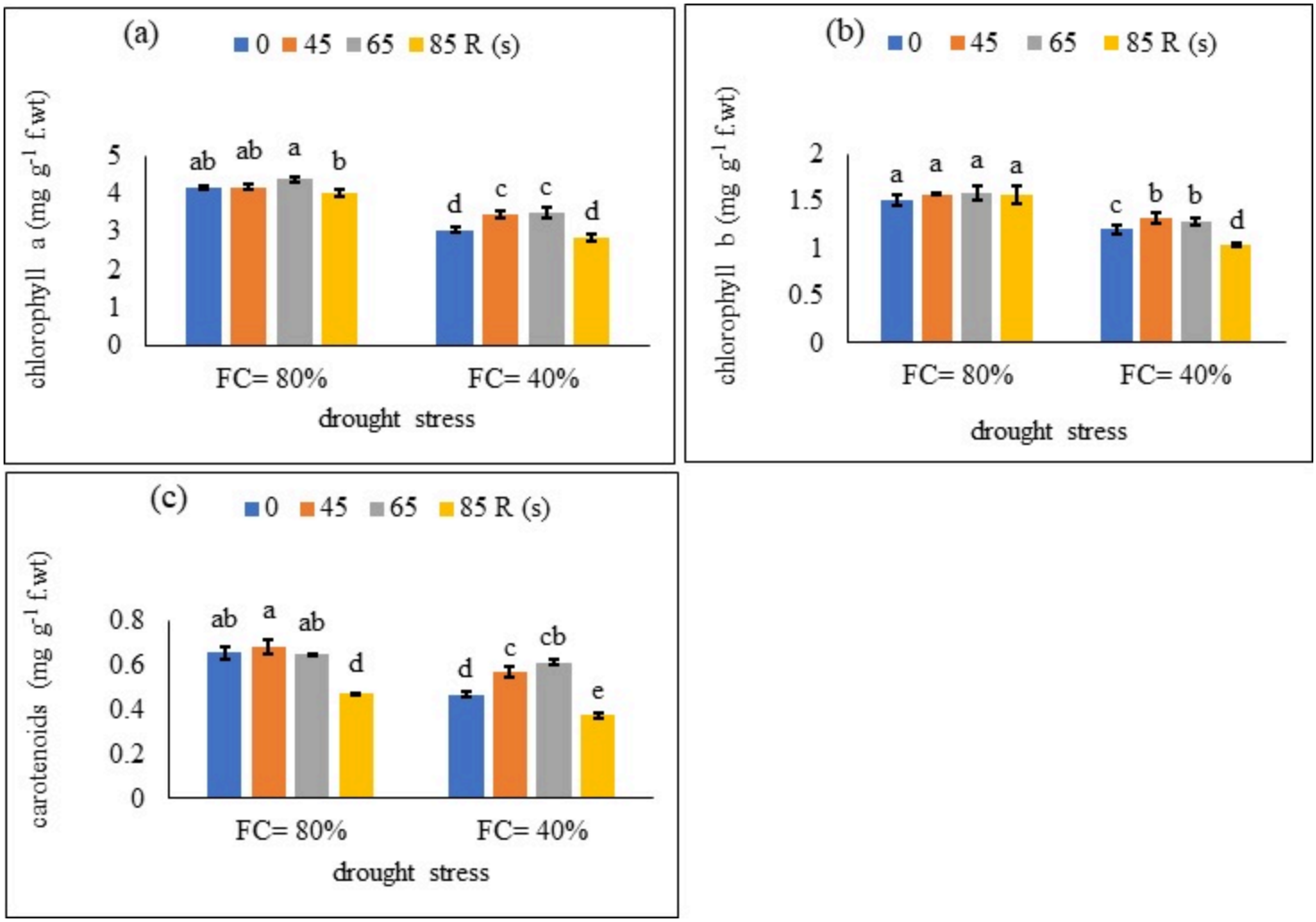

Figure 2

Comparison of the average interaction effect of laser radiation and drought stress in wheat on (a) chlorophyll a, and (b) chlorophyll b, and (c) cartenoids. Legend: R= laser radiation per second, FC=field capacity. Bars with at least one letter in common do not have a statistically significant difference based on Duncan's test at the $p \leq 0.05\%$ or $p \leq 0.01\%$ probability level.

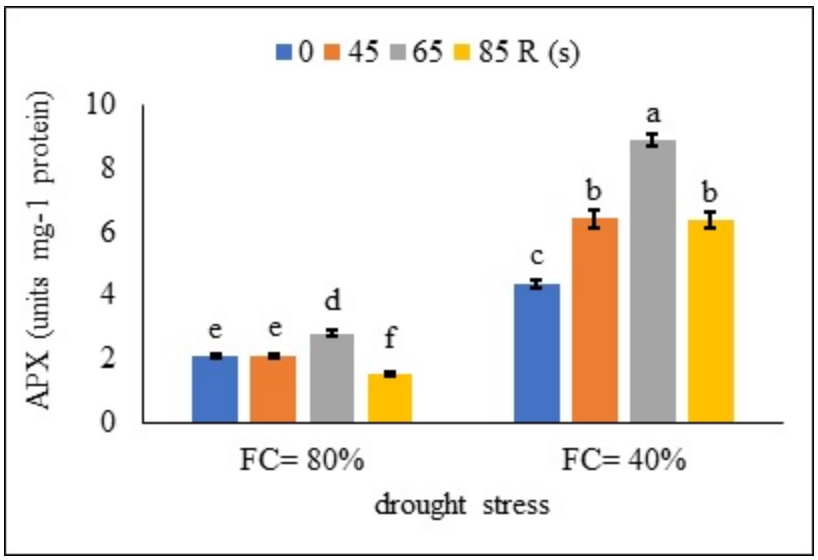

Figure 3

Comparison of the average interaction effect of laser radiation and drought stress in wheat on ascorbate peroxidase (APX) activity. Legend: R= laser radiation per second, FC=field capacity. Bars with at least one letter in common do not have a statistically significant difference based on Duncan's test at the $p \leq 0.05\%$ or $p \leq 0.01\%$ probability level.

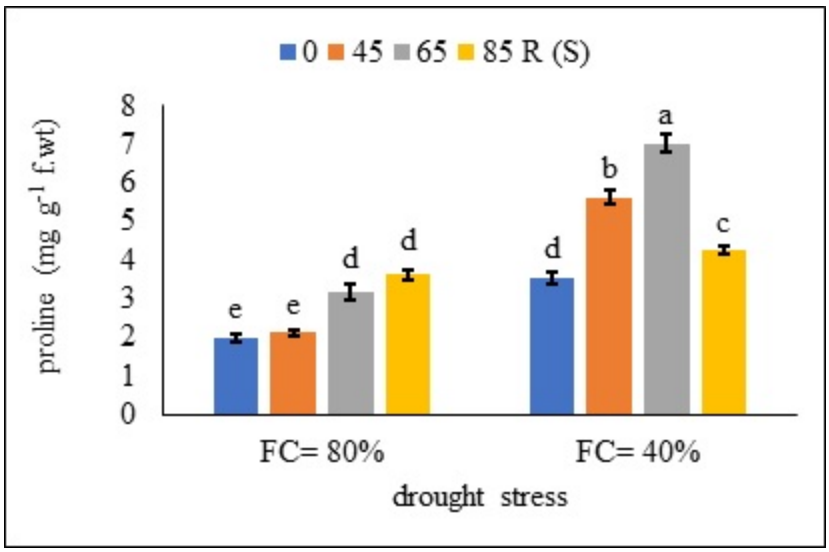

Figure 4

Comparison of the average interaction effect of laser radiation and drought stress in wheat on proline content. Legend: R= laser radiation per second, FC=field capacity. Bars with at least one letter in common do not have a statistically significant difference based on Duncan's test at the $p \leq 0.05\%$ probability level.